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STANDARDIZATION OF CONCENTRATION OF BIO-AGENTS FOR ENHANCED SEEDLING GROWTH OF TOMATO CV. PKM 1

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ABSTRACT

An investigation was carried out with tomato PKM 1 variety to standardize seed biopriming treatment with bio-agents viz., Bascillus subtillus 1, Methylobacterium extorquens AM 1 and Pseudomonas fluorescens Pf 1 with different concentration viz., 1%, 2%, 4%, 6%, 8% and 10% for 9 h duration along with hydropriming for 9 hours. The nonprimed seed formed the control. Seed bioprimed with Pseudomonas fluorescens Pf 1@ 8% concentration for 9 h expressed high speed of germination, germination, root length, shoot length, dry matter production and vigour index which accounted for 18, 14, 26, 46, 13 and 55 % increase respectively, over nonprimed seed.

KEYWORDS: Biopriming, Bio-Agents, Concentration, Seed Germination & Vigour Index

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Original Article

INTRODUCTION

Vegetables constitute a major part in terms of providing food and nutritional security in Indian subcontinent. Vegetables are important sources of minerals, vitamins and other nutrients. The production and productivity of different vegetable crops have been increased significantly in the past twenty years as a result of research and development. Solanaceous vegetables like tomato, brinjal and chilli have high demand in the country. Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops of the world and is widely cultivated throughout the tropical and subtropical countries. Seed treatment with bio-control agents the process often known as bio-priming may serve as an important means of managing many of the soil and seed-borne diseases (Taylor and Harman, 1990). PGPR application through seed priming or soaking the seeds in liquid bacterial suspension starts the physiological processes inside the seed while radicle and plumule emergence is prevented until the seed is sown (Anitha *et al*., 2013). The start of physiological process inside the seed enhances the abundance of PGPR in the spermosphere (Taylor and Harman, 1990). This proliferation of antagonist PGPR inside the seeds is 10 folds higher than the effect of attacking pathogens, which enables the plant to survive against those pathogens (Callan *et al*. 1990). This affirm the increasing the use of bio-control agents for biopriming of seeds.

MATERIALS AND METHODS

Genetically pure seeds of tomato PKM 1 were obtained from Horticultural College and Research Institute, Periyakulam were bioprimed with bio-agents *Bascillus subtillus* 1 *Methylobacterium extorquens* AM 1 *and Pseudomonas fluorescens* Pf 1 obtained from the Department of Agricultural Microbiology and Department of

Plant pathology, Tamil Nadu Agricultural University, Coimbatore.

The laboratory experiment was conducted to standardize an optimum concentration of seed biopriming with bioagents for improved seed germination and seedling growth in completely randomized design (CRD) at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

For priming treatments, the seeds of tomato PKM 1 were soaked in equal the volume of water for 1, 2, 4, 6, 8 and 10 per cent concentration of *Methylobacterium extorquen* AM 1 (liquid), *Bascillus subtillus* 1 (powder), *Pseudomonas fluorescens* Pf 1 (liquid) solutions and in plain water for 9 h separately. The nonprimed seed served as control. After priming, the seeds were dried back to the original moisture content under shade. The laboratory germination test was carried out in four replicates of 100 seeds each from individual treatments using top of paper method.

Speed of Germination

During the germination test period, the emergence of the seedlings with the cotyledons and plumule was counted daily from 2nd day until 14th day of sowing. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the results were expressed in number (Maguire, 1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

Where,

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count

X_n- Number of seeds germinated on n^{th} count

Y_1- Number of days from sowing to first count

Y_2- Number of days from sowing to second count

Y_n- Number of days from sowing to n^{th} count

Germination Percentage

The germination test was conducted in quadruplicate using 100 seeds each with 4 sub replicates of 25 seeds in roll towel method using the paper medium (ISTA, 2009). The test conditions of 25 ± 2°C temperature and 95 ± 3 per cent relative humidity were maintained in germination room. At the end of 14 days, the numbers of normal seedlings were counted and the mean was expressed in percentage.

Root Length

At the time of germination count, ten normal seedlings were selected at random from each replication and used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root. The mean values were calculated and expressed in centimetre.

Shoot Length

The seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the point of attachment of seed to the tip of the leaf and the mean values were expressed in centimetre.

Dry Matter Production

The ten seedlings used for growth measurement were air dried for 6 h and then in a hot air oven maintained at 85 ± 2 °C for 24 h and cooled in desiccator containing silica gel for 30 min. The dry weight of seedlings was recorded using electronic balance.

The mean dry weight of the seedlings was determined and recorded as mg 10seedlings $^{-1}$.

Vigour Index

Vigour index values were computed using the following formulae and the mean values were expressed in whole number (Abdul-Baki and Anderson, 1973). Vigour index = Germination percentage × (Seedling length).

The data obtained from different experiments were analysed for the 'F' test of significance following the methods described by Panse and Sukhatme (1985).

RESULTS AND DISCUSSIONS

Seed Biopriming with *Bacillus Subtillus* 1

Significant variations were observed for speed of germination, germination, root and shoot length, dry matter production and vigour index due to priming treatment with different concentrations of *Bacillus subtillus*. The seeds bioprimed with *Bacillus subtillus* 1 @ 6% concentration for 9 h registered higher speed of germination (8.9) and germination (87%) than nonprimed seed (Figure 1). An increase of 13% was noticed for germination due to seed biopriming with *Bacillus subtillus* 1 @ 6% concentration for 9 h over nonprimed seed. Seedlings from *Bacillus subtillus* 1 @ 6% for 9 h treatment measured the longest root (13.4 cm) and shoot (7.0 cm) while the shortest root and shoot was observed in nonprimed seed (11.3 and 4.4 cm, respectively). Seeds bioprimed with *Bacillus subtillus* 1 @ 6% concentration for 9 h produced the maximum dry matter production (22.1 mg 10 seedlings^{-1}) followed by *Bacillus subtillus* 1@ 4% for 9h (21.6 mg 10 seedlings^{-1}), and the minimum dry matter production was recorded in nonprimed seed (19.7 mg 10 seedlings^{-1}) (Table 1). Seeds bioprimed with *Bacillus subtillus* 1 @ 6% concentration for 9 h also registered m vigour (1774) when compared to other concentrations while the vigour index value of 1161 was recorded in control. (Figure 2).

Table 1: Effect of Seed Biopriming with Different Concentration of *Bacillus Subtillus* 1 on Germination and Seedling Growth in Tomato cv. PKM 1 Seeds

Treatments	Speed of Germination	Root Length (cm)	Shoot Length (cm)	Germination (%) *	Dry Matter Production (mg Seedlings^{-10})	Vigour Index
T_0	7.8	11.3	4.4	74(59.83)	19.7	1161
T_1	8.0	11.9	4.7	76(60.72)	20.7	1261
T_2	8.4	12.2	5.4	80(63.40)	21.3	1408
T_3	8.6	12.4	5.7	82 (64.95)	21.4	1484
T_4	8.8	12.7	6.6	85 (67.63)	21.6	1640
T_5	8.9	13.4	7.0	87 (69.70)	22.1	1774
T_6	8.5	12.3	5.8	84(66.42)	21.1	1520
T_7	8.4	12.0	5.6	83(65.65)	20.9	1460
Mean	**8.4**	**12.2**	**5.6**	**81(64.16)**	**21.1**	**1441**
SEd	0.10	0.16	0.09	0.80	0.24	18.25
CD (P=0.05)	0.21	0.34	0.19	1.65	0.51	37.66

* - Values in parentheses are arcsine transformed values

Treatment Details

T_0 - Nonprimed seed

T_1 - Hydropriming for 9 h

T_2 - *Bacillus subtillus* 1@ 1% concentration for 9 h

T_3 - *Bacillus subtillus* 1@ 2% concentration for 9 h

T_4 - *Bacillus subtillus* 1@ 4% concentration for 9 h

T_5 - *Bacillus subtillus* 1@ 6% concentration for 9 h

T_6 - *Bacillus subtillus* 1@ 8% concentration for 9 h

T_7 - *Bacillus subtillus* 1@ 10% concentration for 9 h

Seed Biopriming with *Methylobacterium Extorquens* AM 1

The speed of germination, germination, root and shoot length, dry matter production and vigour index were significantly influenced by biopriming treatment with different concentration of *Methylobacterium extorquens* AM 1. The results indicated the better performance of *Methylobacterium extorquens AM* 1 @ 4% concentration for 9 h with respect to speed of germination (8.8). The lowest speed of germination of 7.8 was noticed in nonprimed seed which was on par with hydropriming for 9 h (8.0). Seeds primed with *Methylobacterium extorquens AM* 1 @ 4% concentration for 9 h also recorded higher germination (85%) which showed an increase of 11% over nonprimed seed (Figure 1). Seeds bioprimed with *Methylobacterium extorquens* AM 1@ 4% concentration for 9 h measured the longest root (13.5 cm) and shoot (6.1 cm) compared to nonprimed seed (11.3 and 4.4 cm, respectively). The biopriming involving *Methylobacterium extorquens* AM 1@ 4% concentration for 9 h registered higher dry matter production (22.8 mg 10 seedlings^{-1}) followed by *Methylobacterium extorquens* AM 1@ 2% concentration for 9 h (21.8 mg 10 seedlings^{-1}). The dry matter production of *Methylobacterium extorquens* AM 1@ 1% concentration for 9 h, hydroprimed seed for 9 h and nonprimed seed was 20.3 mg 10 seedlings^{-1}, 20.0 mg 10 seedlings^{-1} and 19.7 mg 10 seedlings^{-1} which were on a par with each other (Table 2). Compared to other concentrations and nonprimed seeds (1161), the *Methylobacterium extorquens* AM 1 @ 4% for 9 h registered maximum vigour index value (1666) (Figure 2).

Table 2: Effect of Seed Biopriming with Different Concentration of *Methylobacterium Extorquens* AM 1 on Germination and Seedling Growth in Tomato cv. PKM 1 Seeds

Treatments	Speed of Germination	Root Length (cm)	Shoot Length (cm)	Germination (%) *	Dry Matter Production (mg seedlings^{-10})	Vigour Index
T_0	7.8	11.3	4.4	74(59.83)	19.7	1161
T_1	8.0	11.9	4.7	76(60.05)	20.0	1261
T_2	8.3	12.1	5.1	80(63.40)	20.3	1376
T_3	8.5	13.1	5.5	84(66.42)	21.8	1562
T_4	8.8	13.5	6.1	85 (67.63)	22.8	1666
T_5	8.4	12.6	5.4	84(66.42)	21.4	1512
T_6	8.4	12.4	5.2	83(65.65)	21.1	1460
T_7	8.3	12.0	5.1	82 (64.95)	20.1	1402
Mean	**8.3**	**12.3**	**5.1**	**81(64.95)**	**20.9**	**1409**

Table 2: Contd.,						
SEd	0.12	0.18	0.05	1.04	0.21	21.03
CD (P=0.05)	0.24	0.37	0.11	2.15	0.43	43.41

* - Values in parentheses are arcsine transformed values **Treatment details**

T_0 - Nonprimed seed

T_1 - Hydropriming for 9 h

T_2 - *Methylobacterium extorquens* AM 1 @ 1% concentration for 9 h

T_3 - *Methylobacterium extorquens* AM 1 @ 2% concentration for 9 h

T_4 - *Methylobacterium extorquens* AM 1 @ 4% concentration for 9 h

T_5 - *Methylobacterium extorquens* AM 1 @ 6% concentration for 9 h

T_6 - *Methylobacterium extorquens* AM 1 @ 8% concentration for 9 h

T_7 - Methylobacterium extorquens AM 1 @ 10% concentration for 9 h

Seed Biopriming with *Pseudomonas Fluorescens* Pf1

Significant differences were observed for speed of germination, germination, root and shoot length, dry matter production and vigour index due to priming treatment with different concentration of *Pseudomonas fluorescens* Pf 1. The seeds bioprimed with *Pseudomonas fluorescens* Pf 1 @ 8% for 9h registered higher speed of germination (9.2) and germination (88%) than nonprimed seed (Figure 1). An increase of 14% was noticed for germination due to *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h biopriming over nonprimed seed (Table 3). Seedlings from the seeds bioprimed with *Pseudomonas fluorescens* Pf 1@ 8% concentration for 9 h measured the longest root (14.2 cm) and shoot (6.2 cm) and the shortest root and shoot was observed in nonprimed seed (11.3 and 7.8 cm, respectively). Seeds bioprimed with *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h produced the maximum dry matter production (22.2 mg 10 seedlings^{-1}) followed by *Pseudomonas fluorescens* Pf 1 @ 6% concentration for 9 h (21.9 mg 10 seedlings^{-1}) which were on par with each other. The dry matter production was the minimum in nonprimed seed (19.7 mg 10 seedlings^{-1}). *Pseudomonas fluorescens* Pf 1@ 8% concentration for 9 h treatment also registered more vigour (1795) when compared to other treatments. The vigour index value of control was only 1161 (Figure 2).

The present study, revealed that the seed biopriming with *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h was found to be the best biopriming treatment for improving the seed germination (Figure 3) and seedling vigour of tomato cv. PKM 1(Figure 4).

Table 3: Effect of Seed Biopriming with Different Concentration of *Pseudomonas Fluorescens* Pf 1 on Germination and Seedling Growth in Tomato cv. PKM 1 Seeds

Treatments	Speed of Germination	Root Length (cm)	Shoot Length (cm)	Germination (%) *	Dry Matter Production (mg seedlings^{-10})	Vigour Index
T_0	7.8	11.3	4.4	74(59.83)	19.7	1161
T_1	8.0	11.9	4.7	76(60.72)	20.0	1261
T_2	8.5	12.2	5.7	79(62.82)	21.2	1414
T_3	8.8	12.4	5.8	84(66.42)	21.4	1528
T_4	9.0	13.1	5.9	85 (67.63)	21.7	1615

Table 3: Contd.,						
T_5	9.1	14.1	6.0	85 (67.63)	21.9	1708
T_6	9.2	14.2	6.2	88 (70.04)	22.2	1795
T_7	8.5	12.5	5.8	77(61.69)	21.1	1409
Mean	**8.6**	**12.7**	**5.5**	**81(64.16)**	**21.15**	**1486**
SEd	0.10	0.14	0.06	1.04	0.21	22.60
CD (P=0.05)	0.21	0.30	0.14	2.15	0.43	46.65

* - Values in parentheses are arcsine transformed values

Treatment Details

T_0 - Nonprimed seed

T_1 - Hydropriming for 9 h

T_2 - *Pseudomonas fluorescens* Pf 1 @ 1% concentration for 9 h

T_3 - *Pseudomonas fluorescens* Pf 1 @ 2% concentration for 9 h

T_4 - *Pseudomonas fluorescens* Pf 1 @ 4% concentration for 9 h

T_5 - *Pseudomonas fluorescens* Pf 1 @ 6% concentration for 9 h

T_6 - *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h

T_7 - *Pseudomonas fluorescens* Pf 1 @ 10% concentration for 9 h

Several studies showed that biopriming was very useful for better seed germination, seedling vigour and disease management in many crops viz., groundnut (Malathi and Doraisamy, 2004), pea (Mohamedy and Baky, 2008), maize (Nayaka *et al.,* 2008), chilli, tomato and brinjal (Someshwar and Sitansu, 2010). Higher germination and enhanced seedling establishment was also obtained through seed priming with PGPR (Anitha *et al*., 2013) and PGPR inoculation increased the stress resistance and production of the crops; including tomato (Almaghrabi *et al.,* 2013), wheat (Jaderlund *et al.,* 2008; Chakraborty *et al.,* 2013; Nadeem *et al.* 2013; Islam *et al.* 2014; Kumar *et al.* 2014), rice (Bal *et al.,* 2013; Jha *et al.,* 2013; Lavakush *et al.,* 2014), soybean (Masciarelli *et al.,* 2014), groundnut (Paulucci *et al.,* 2015), broad bean (Younesi and Moradi, 2014), maize (Rojas-Tapias *et al.,* 2012) and chickpea (Patel *et al.,* 2012).

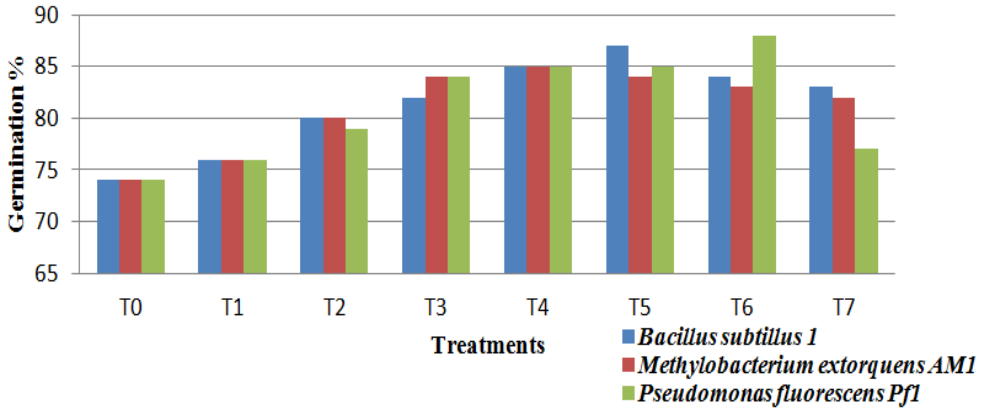

Figure 1: Effect of Biopriming of Seeds with Different Concentration of *Bacillus Subtillus* 1, *Methylobacterium Extorquens* AM 1, Pseudomonas *Fluorescens* Pf 1 on Germination % in Tomato cv. PKM 1 Seeds

Bacillus sp. and *Pseudomonas* sp. are used as biocontrol agent against diseases that are soilborne and airborne. These bacteria may produce compounds that are antibiosis as chitinase enzyme that can hydrolyze the cell walls of fungi (Wang and Chang, 1997), siderophores and other antibiotics that can inhibit the development of pathogens (Habazar and Yaherwandi, 2006). Biopriming has been practiced and explained by different researchers (Callan *et al.,* 1991; Bennett *et al.,* 2009; Moeinzadeh *et al.,* 2010; Chakraborty *et al.,* 2011; Sharifi, 2011; Sharifi and Khavazi, 2011; Gururani *et al.,* 2012; Mirshekari *et al.,* 2012; Sharifi, 2012). (Bennett, 1998) reported that bio-osmopriming can significantly enhance the uniformity of seed germination and plant growth traits when associated with bacterial coating and better stand establishment.

Studies also revealed that the possible mechanism by which Methylobacterium promotes plant growth includes production of phytohormones such as indole 3 acetic acid (IAA), cytokinin or vitamins (Basile *et al.,* 1985; Koenig *et al.,* 2002, Lidstrom and Chisto serdova, 2002), enhancement of nitrogen metabolism in plants by bacterial urease (Holland and Polacco, 1992), formation of root nodules in legumes (Sy *et al.,* 2001), nitrate reductase activity and 1 amino cyclopropane 1carboxylate deaminase activity (Madhaiyan *et al.,* 2006). The improvement in seedling vigour parameters due to seed biopriming with *Methylobacterium extorquens* AM 1 4 % concentration for 9 h could be possibly because of the production of germination accelerating and growth promoting substances. Similar findings have also reported an increase in the percentage of seed germination, seed vigour and dry matter accumulation (Madhaiyan *et al.,* 2004; Madhaiyan *et al.,* 2005; Lee *et al.,* 2006) with inoculation of strains of Methylobacterium. Higher speed of germination, germination, root length, shoot length, dry matter production and vigour index in *Pseudomonas fluorescens* Pf 1 8% concentration for 9 h which accounted for 18, 14, 26, 46, 13 and 55 % increase over nonprimed seed in this present study is in agreement with the findings of Hatayama (2005) who reported that PGPR such as *Bacillus* sp. and *Pseudomonas* sp. are capable of providing a direct influence that can trigger the growth of plants (biostimulant), while the indirect effect that the bacteria is able to inhibit the growth of harmful microbes.

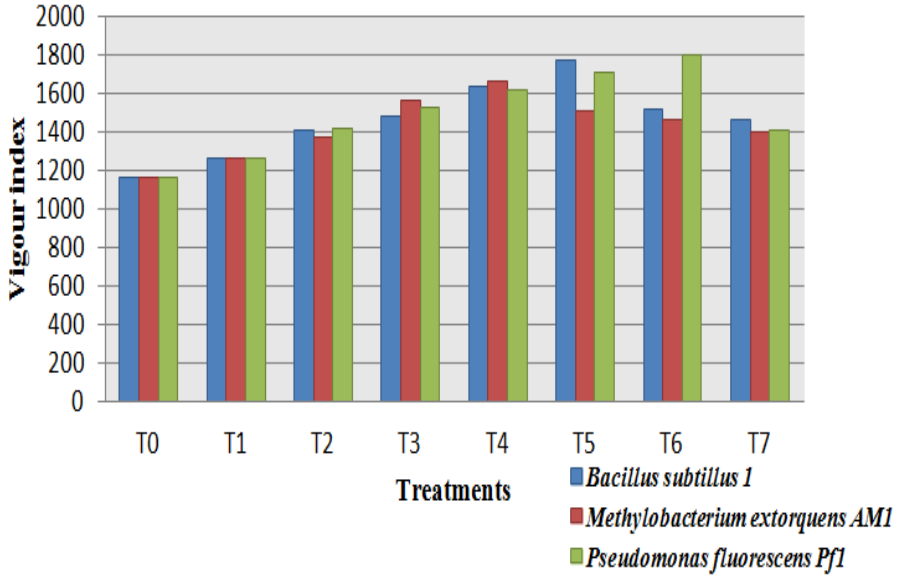

Figure 2: Effect of Biopriming of Seeds with Different Concentration of *Bacillus Subtillus* 1, *Methylobacterium Extorquens* AM 1, *Pseudomonas Fluorescens* Pf 1 on Vigour Index in Tomato cv. PKM 1 Seeds

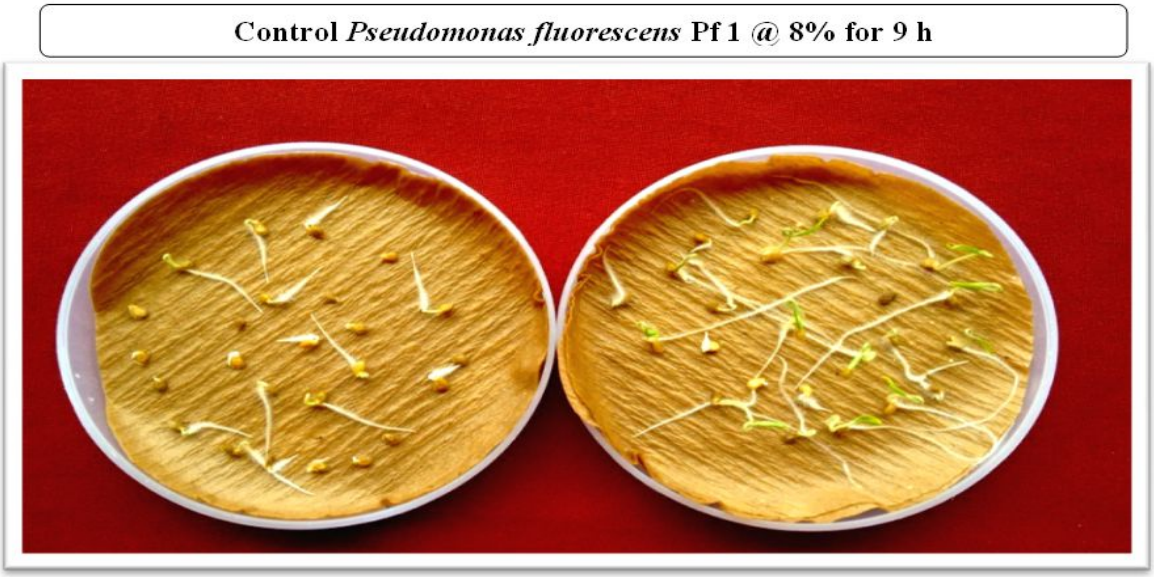

Figure 3: Effect of Biopriming of Seeds with *Pseudomonas Fluorescens* Pf 1 @ 8% for 9 H on Speed of Germination in Tomato cv. PKM 1 Seeds

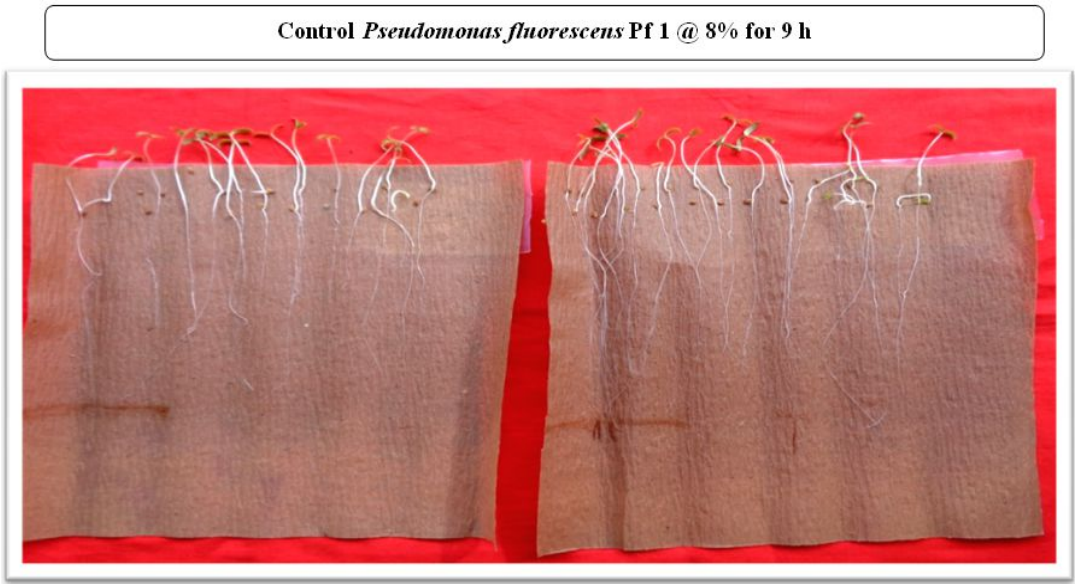

Figure 4: Effect of Biopriming of Seeds with *Pseudomonas Fluorescens* Pf 1 @ 8% for 9 H on Germination % for Tomato Cv. PKM 1 Seeds

CONCLUSIONS

The application of bacteria to small seeded crops which can imbibe the bacterial suspension results in entry of bacteria inside the seed. Biopriming gives control against several root rot diseases and also enhances seed vigour so can be used commercially as an alternative to fungicides. It is summarized from this study that, seed biopriming with liquid *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h or powder *Bacillus subtillus* 1 @ 6% concentration for 9 h or liquid *Methylobacterium extorquens* AM 1 @ 4% for 9 h was found to be the best biopriming treatment for improving the seed germination and production of vigourous diseases free seedling vigour of tomato.

REFERENCES

1. *Abdul-Baki, A.A., & Anderson, J.D. (1973). Vigour deterioration of soybean seeds by multiple criteria. Crop Sci. 13:630-633.*
2. *Almaghrabi O.A., Massoud, S.I. & Abdelmoneim, T.S. (2013). Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J Biol Sci 20: 57–61.*
3. *Anitha, D., Vijaya, T & Reddy, N.V. (2013). Microbial endophytes and their potential for improved bioremediation and biotransformation: a review. Indo Am J Pharmaceutical Res 3: 6408–6417.*
4. *Bal, H.B., Nayak, L., & Das, S. (2013). Isolation of ACC deaminase PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil, 366: 93–105.*
5. *Basile, D.V., Basile, M.R., Li Q. Y., & Corpe, W. A. (1985). Vitamin B12 stimulated growth and development of Jungermannia leiantha Grolle and Gymnocolea inflate (Huds.) Dum (Hepaticae). Bryologist 88, 77-81.*
6. *Bennett, A.J, Mead, A., & Whipps, J.M. (2009). Performance of carrot and onion seed primed with beneficial microorganisms in glasshouse and field trials. Biol Control 51: 417-426.*
7. *Bennett, M.A. (1998). The use of biologicals to enhance vegetable seed quality. Seed Technol 20: 198-208.*
8. *Bhagat, S., & Pan, S. (2010). Cultural and phenotypic characterization of Trichoderma spp from Andaman and Nicobar Islands J. Mycol. Pl. Pathol. 40(1): 145-157.*
9. *Callan, N.W, Mathre, D.E., & Miller, J.B. (1991). Field performance of sweet corn seed bio-primed and coated with Pseudomonas fluorescens AB254. Hortscience 26: 1163–1165.*
10. *Callan, N.W., Mathre, D.E., & Miller, J.B. (1990). Bio-priming seed treatment for control of Pythium ultimum pre emergence damping-off in sh-2 sweet corn. Plant Dis 74: 368-372.*
11. *Chakraborty, A.P, Dey, P., & Chakraborty, B. (2011). Plant growth promotion and amelioration of salinity stress in crop plants by a salt-tolerant bacterium. Rec Res Sci Technol 3: 61-70.*
12. *Chakraborty, U., Chakraborty, B.N, & Chakraborty, A.P. (2013). Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J Microbiol Biotechnol 29: 789–803.*
13. *Gururani, M.A., Upadhyaya, C.P., & Baskar, V. (2012) Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in Solanum tuberosum through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. J Plant Growth Regul 32: 245-258.*
14. *Habazar, T., & Yaherwandi., (2006). Pengendalian Hayati Hama dan Penyakit Tumbuhan. Andalas Press. Padang.*
15. *Hatayama, K., Kawai, S., Shoun, H., Ueda, Y., & Nakamura, A., (2005). Pseudomonas azotiFigureens sp. nov., A Novel Nitrogen-Fixing Bacterium Isolated From A Compost Pile. International Journal of Systematic and Evolutionary Microbiology 55, 1539-1544.*
16. *Holland, M. A. & Polacco, J. C. (1992). Urease null and hydrogenase null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. Plant Physiol 98:942-948.*
17. *Islam, F., Yasmeen, T., & Ali, Q. (2014). Influence of Pseudomonas aeruginosa as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotox Environ Safe 104: 285–293.*
18. *ISTA (2009). International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland.*

19. Jaderlund, L., Arthurson, V., & Granhall, U. (2008). Specific interactions between arbuscular mycorrhizal fungi and plant growth-promoting bacteria: as revealed by different combinations. FEMS Microbiol Lett 287: 174–180.

20. Jha, A., Saxena, J., & Sharma, V. (2013). An investigation on phosphate solubilization potential of agricultural soil bacteria as affected by different phosphorus sources, temperature, salt and pH. Commun Soil Sci Plant Analysis 44: 2443–2458.

21. Koenig, R. L., R. O. Morris & Polacco, J. C. (2002). tRNA is the source of low level trans zeatin production in Methylobacterium spp. J Bacteriol 184, 1832-1842.

22. Kumar, A., Maurya, B.R & Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (Triticum aestivum L.). Biocatalysis Agric Biotechnol 3: 121–128.

23. Lavakush, Y.J, Verma, J.P, & Jaiswal, D.K. (2014) Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa). Ecol Eng 62: 123–128.

24. Lee, H. S., Madhaiyan, M., Kim, C. W., Choi, S. J., Chun, K. Y. & Sa, T. M. (2006). Physiological enhancement of early growth of rice seedlings (Oryza sativa L) by production of phytohormone of N_2 -fixing methylotrophic isolates. Biol. Fer. Soils 42:402-408.

25. Lidstrom, M. E. & Chistoserdova, L. (2002). Plants in the pink: cytokinin production by Methylobacterium. J Bacteriol 184, 1818.

26. Madhaiyan, M., Poonguzhali, S., Lee, H. S., Hari, K., Sundaram, S. P., & Sa, T. M. (2005). Pink pigmented facultative methylotrophic bacteria accelerate germination, growth and yield of sugarcane clone Co86032 (Saccharum officinarum L). Biol. Fertil. Soils 41:350-358

27. Madhaiyan, M., Poonguzhali, S., Ryu J. H., &. Sa, T. M. (2006).Regulation of ethylene levels in canola (Brassica campestris) by 1 aminocyclopropane 1 carboxylate deaminase containing Methylobacterium fujisawaense. Planta 224:268-278.

28. Madhaiyan, M., Poonguzhali, S.,. Senthilkumar, M., Seshadri, S., Chung, H. Y., Sundaram, S., & Sa, T. M. (2004). Growth promotion and induction of systemic resistance in rice cultivar Co 47 (Oryza sativa L) by Methylobacterium sp. Bot. Bull. Acad. Sin. 45:315-324.

29. Maguire, J.D.(1962). Speed of germination - Aid in selection and evaluation of seedling emergence and vigour. Crop Sci. 2:176-177.

30. Malathi, P. & Doraisamy, S. (2004): Effect of seed priming with Trichoderma on seed borne infection of Macrophomina phaseolina and seed quality in groundnut. Annals-of-Plant-Protection-Sciences. 12 (1):87-91.

31. Masciarelli, O., Llanes, A., & Luna, V. (2014) A new PGPR co-inoculated with Bradyrhizobium japonicum enhances soybean nodulation. Microbiol Res 169: 609–615.

32. Mirshekari, B., Hokmalipour, S., & Sharifi, R.S. (2012) Effect of seed biopriming with plant growth promoting rhizobacteria (PGPR) on yield and dry matter accumulation of spring barley (Hordeum vulgare L.) at various levels of nitrogen and phosphorus fertilizers. J Food Agri Environ 10: 314-320.

33. Moeinzadeh, A., Sharif-Zadeh. F., & Ahmadzadeh. M. (2010) Biopriming of sunflower (Helianthus annuus L.) seed with Pseudomonas fluorescens for improvement of seed invigoration and seedling growth. Aust J Crop Sci 4: 564.

34. Mohamedy, E. I. R. S. R. & Baky A. E. l. M. M. H. (2008): Evaluation of different types of seed treatment on control of root rot disease, improvement growth and yield quality of pea plant in nobaria province. Research Journal of Agriculture and Biological Sciences, 4 (6): 611-622.

35. *Nadeem, S.M., Zaheer, Z.A., & Naveed, M. (2013) Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions. Ann Microbiol 63: 225–232.*
36. *Nayaka, S. R., Niranjana, A. C., Uday Shankar, S., Niranjanraj, M. S., Reddy, H. S., Prakash, C. N., & Mortensen (2008). Archives Phytopath. and Plant Prot., 43 (3): 264 – 282.*
37. *Panse, V. G. &. Sukatme. P. V. (1985). Statistical methods for agricultural workers. ICAR publication, New Delhi, 359.*
38. *Patel H.A., Patel R.K., & Khristi S.M. (2012) Isolation and characterization of bacterial endophytes from Lycopersicon esculentum plant and their plant growth promoting characteristics. Nepal J Biotechnol 2: 37-52.*
39. *Paulucci N.S., Gallarato L.A., & Reguera, Y.B. (2015) Arachis hypogaea PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity. Microbiol Res 173: 1–9.*
40. *Rojas-Tapias, D., Moreno-Galván, A., & Pardo-Díaz, S. (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Appl Soil Ecol 61: 264-272.*
41. *Sharifi, R..S & Khavazi, K. (2011) Effects of seed priming with plant growth promotion rhizobacteria (PGRP) on yield and yield attribute of maize (Zea mays L.) hybrids. J Food Agri Environ **9**: 496-500.*
42 *.Sharifi, R.S. (2011) Study of grain yield and some of physiological growth indices in maize (Zea mays L.) hybrids under seed biopriming with plant growth promoting rhizobacteria (PGPR). J Food Agri Environ 189: 3-4.*
43. *Sharifi, R.S. (2012) Study of nitrogen rates effects and seed biopriming with PGPR on quantitative and qualitative yield of Safflower (Carthamus tinctorius L.). Tech J Eng Appl Sci 2: 162-166.*
44. *Sy, A.,. Giraud, E., Jourand, P., Garcia, N., Willems, A., Lajudie, P. de., Prin,. Y. Neyra, M., & Gillis, M. (2001). Methylotrophic Methylobacterium bacteria nodulate and fix nitrogen in symbiosis with legumes. J Bacteriol 183:214-220.*
45. *Taylor, A.G & Harman, G.E. (1990) Concept and technologies of selected seed treatments. Ann Rev Phytopathol 28: 321-339.*
46. *Wang, S.L., & Chang, W.T. (1997). Purification and Characterization of Two Bifungsional Chitinases/ Lysozymes Extracellularly Produced by Pseudomonas aeruginosa K-187 in a Shrimp and Crab Shell Powder Medium, Appl. and Environ. Microbial. 63(2), 380–386.*
47. *Younesi, O., & Moradi, A. (2014). Effects of plant growth-promoting rhizobacterium (PGPR) and arbuscular mycorrhizal fungus (AMF) on antioxidant enzyme activities in salt-stressed bean (Phaseolus vulgaris L.). Agriculture (Pol'nohospodárstvo). 60: 10−21.*